

Bifurcation analysis of a model of the budding yeast cell cycle

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Abstract

We study the bifurcations of a set of nine nonlinear ordinary differential equations that describe the regulation of the cyclin-dependent kinase that triggers DNA synthesis and mitosis in the budding yeast, *Saccharomyces cerevisiae*. We show that Clb2-dependent kinase exhibits bistability (stable steady states of high or low kinase activity). The transition from low to high Clb2-dependent kinase activity is driven by transient activation of Cln2-dependent kinase, and the reverse transition is driven by transient activation of the Clb2 degradation machinery. We show that a four-variable model retains the main features of the nine-variable model. In a three-variable model exhibiting birhythmicity (two stable oscillatory states), we explore possible effects of extrinsic fluctuations on cell cycle progression.

Keywords: regulatory networks, cellular control, fluctuations

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I. INTRODUCTION

The cell cycle is the sequence of events by which a growing cell replicates all its components and divides them evenly between two daughter cells [1, 2, 3]. Many theoreticians have understood the cell cycle as a periodic process driven by a biochemical limit cycle oscillator [4, 5, 6]. However, a growing body of experimental and theoretical evidences indicates that the eukaryotic cell cycle is a toggle switch between two stable steady states, controlled by checkpoints [7, 8, 9, 10, 11]. This point of view was adopted by Chen et al. [9] in a recent mathematical model of the budding yeast cell cycle, and bistability in the yeast cell cycle control system has been confirmed recently by experiments from Cross's laboratory [10].

Bifurcation theory is a mathematical tool for characterizing steady state and oscillatory solutions of a system of nonlinear differential equations (ODE's) [12, 13]. The goal of this work is a detailed bifurcation analysis of Chen's model. Our bifurcation analysis supplements the numerical simulation carried out by Chen et al. and clarifies their quantitative comparisons between experiment and theory [10]. In addition, bifurcation theory helps us to identify control modules within Chen's complicated model, thereby bringing some new insights to the yeast cell cycle control mechanism. A more thorough understanding of cell cycle control in yeast can be very helpful in future efforts to model mammalian cell cycle controls [14].

This paper is organized as follows. In Section II, we give a brief introduction to the budding yeast cell cycle. In Section III, we introduce Chen's model and present its one-parameter bifurcation diagram. In Section IV, we study saddle node bifurcations in Chen's model in order to provide a rigorous foundation for interpreting Cross's experiment on bistability of the control system [10]. In Section V, we propose a reduced model with four time-dependent variables, which retains the main dynamical characteristics of the extended model. We characterize this model using two-parameter bifurcation diagrams. In Section VI, we further reduce Chen's model to three variables and demonstrate that the abbreviated model displays bifurcations and birhythmicity similar to more complex models [8, 15, 16]. In Section VII, we use the three-variable model to study effects of extrinsic fluctuations. The closing section is devoted to discussion. The nine-variable mathematical model and its parameters are given in the Appendix.

II. A BRIEF INTRODUCTION TO THE BUDDING YEAST CELL CYCLE

Cell cycle phases. The cell cycle is the process by which one cell becomes two. The most important events in the cell cycle are replication of the cell's DNA and separation of the replicated DNA molecules to the daughter cells. In eukaryotic cells, these events (replication and separation) occur in temporarily distinct stages (S phase and M phase, respectively). S and M phase are separated in time by gaps called G1 and G2 phases.

During S phase ("synthesis"), double-stranded DNA molecules are replicated to produce pairs of sister chromatids. During M phase ("mitosis"), sister chromatids are separated so that each daughter cell receives a copy of each chromosome. The G1 checkpoint mechanism controls the initiation of S phase, and a G2 checkpoint mechanism controls entry in M phase. A mitotic checkpoint controls the transition from M phase back to G1 phase. The checkpoints monitor cell size, DNA damage and repair, DNA replication, and chromosome alignment on the mitotic spindle.

Molecular controls of budding yeast cell cycle. Based on current knowledge about the molecular components controlling progression through the budding yeast cell cycle, a molecular wiring diagram was proposed by Chen et al. [9]. A slightly simplified version of their diagram is presented in Figure 1. The molecular components can be divided into four groups: cyclins, inhibitors, transcription factors, and proteolytic machinery.

There are two families of cyclins in Figure 1: Cln's and Clb's [36]. These cyclins combine with kinase subunits (Cdc28) to form active cyclin-dependent kinase heterodimers that trigger cell cycle events (Cdc28/Cln2 initiates budding, Cdc28/Clb5 initiates DNA synthesis, Cdc28/Clb2 initiates mitosis). Cdc28 subunits are in constant, high abundance throughout cell cycle; hence, the activity of Cdc28/cyclin heterodimers is controlled by the availability of cyclin subunits. For this reason, Cdc28 is not shown in Figure 1; only the cyclin subunits are specified. (Each cyclin molecule is understood to have a Cdc28 partner.)

Sic1 (in Figure 1) is a cyclin-dependent kinase inhibitor: it binds to Cdc28/Clb dimers to form inactive trimers (Cdc28/Clb/Sic1). Sic1 does not bind to or inhibit Cdc28/Cln dimers.

Mcm1, MBF, SBF and Swi5 are transcription factors for synthesis of Clb2, Clb5, Cln2 and Sic1, respectively.

The degradation of these proteins is regulated by a ubiquitination pathway. Proteins destined for degradation are first labeled by attachment of multiple ubiquitin molecules.

Ubiquitin moieties are attached to Clb2 and Clb5 by the APC (anaphase promoting complex) in conjunction with either Cdc20 or Hct1. Sic1 is ubiquitinated by a different mechanism (the “SCF”), which (unlike the APC) requires that its substrates be phosphorylated.

Budding yeast cells progress through the division cycle as the levels of the species in Figure 1 come and go. Thus the problem of cell cycle control is to understand the temporal fluctuations of these species. Because the species in Figure 1 are directly or indirectly interacting with all other species, simultaneous determination of their fluctuating concentrations require a precise mathematical model. Using mass action and Michaelis-Menten rate laws, the complex wiring diagram in Figure 1 can be converted into ordinary differential equations, and from them the molecular levels can be computed [9].

III. A BIFURCATION DIAGRAM OF CHEN’S MODEL

The model proposed by Chen et al. [9] includes about a dozen ODE’s and eleven algebraic equations with more than 50 parameters. (Refer to [9] for a complete description of the wiring diagram and a derivation of the mathematical model, as well as for estimates of the rate constants in the model.)

In the appendix we present a reduced version of Chen’s model to be used in this paper for bifurcation analysis. From the original model, we drop the target variables (spindle and bud formation, and DNA synthesis) because they are decoupled from the rest of the model. We reserve mass as the principal bifurcation parameter. We use the same parameter values as Ref. [9], and they are presented in the appendix, Table I.

Using the software package AUTO [17], we created a one-parameter bifurcation diagram (Figure 2) of the budding yeast cell cycle model, Eqn. (A1-A20), for parameter values given in Table I. Two saddle node bifurcations, at $M \approx 0.97$ and $M \approx 0.6$, connect the stable steady states in Figure 2. There is also a subcritical Hopf bifurcation on the upper branch of steady states at $M \approx 0.82$ from which a branch of unstable limit cycles originates. These unstable oscillations disappear at an infinite-period saddle loop (SL) bifurcation near $M \approx 0.73$. A second branch of limit cycle oscillations, shown by filled circles that disappear at a different SL bifurcation point ($M \approx 0.78$), are stable.

The stable steady states (solid line) at values of $[Clb2] < 10^{-3}$ represent the G1 phase of the cell cycle. The stable oscillatory states (filled circles) represent autonomous progression

through S, G2 and M phases of the cell cycle and then back into S phase.

To get a full picture of cell cycle events, we must combine the dynamics of the cyclin-dependent kinase “engine” (as summarized in Figure 2) with equations for cell growth and division (changes in cell mass, M). To this end, we supplemented Eqn. (A1-A20) with an equation for mass growth, $M(t) = M(0)e^{\mu t}$, or, in differential form, $\dot{M} = \mu M$, and a rule for cell division (M reset to fM whenever Clb2-dependent kinase activity drops below 0.005). Following Chen et al. [9] we choose $f = 0.0043$ because budding yeast cells divide asymmetrically.

With these changes, we compute a solution of the full system, Eqn. (A1-A20) plus the dynamics of M , and plot the resulting “trajectory of motion” on the bifurcation diagram (the red line in Figure 2). This trajectory shows that the control system stays in the G1 phase if $M < 0.97$. As M increases further, the control system is captured by the stable limit cycle. As a result, $[\text{Clb2}]_T$ increases abruptly, driving the cell through S phase into M phase, then $[\text{Clb2}]_T$ drops below 0.005, causing the cell to divide and the control system to return to the stable G1 state.

This bifurcation diagram of the Chen et al. model exhibits the same features of cell cycle models of frog eggs [15] and fission yeast [16], namely, saddle node bifurcations associated with stable and unstable oscillations. Yet, there are subtle differences in these bifurcation diagrams. In the frog egg and fission yeast models, the large amplitude stable limit cycles end at a saddle-node invariant-circle (SNIC) bifurcation, not a SL bifurcation. In our case stable oscillations coexist with the stable steady states over a small range of mass values ($0.78 < M < 0.95$). However, when the budding yeast cell cycle model is supplemented by the mass growth equation, such differences seem unimportant.

IV. SADDLE-NODE BIFURCATIONS DRIVEN BY CLN2 AND CDC20

Recently, Cross et al. [10] experimentally confirmed bistability in activity of Clb2-dependent kinase in budding yeast cells. It is interesting to mention that this result was predicted by a schematic sketch (Figure 9 of Ref. [9]) intuitively drawn from interrelations of Cdc28/Clb2 with the G1 phase cyclin Cln2 and the APC specificity factor Cdc20. We confirm this informal prediction of Chen et al. by a rigorous bifurcation analysis of their model.

In their experimental work, Cross et al. constructed a strain that under different experimental conditions may lack activities of Cln2 or Cdc20 or both. (To be precise, Cross et al. used Cln3 in place of Cln2, but that technical detail makes no difference to our analysis.) By manipulating the activities of Cln2 or Cdc20, they found that [Clb2] can be either in high or low, depending on initial conditions. In terms of bifurcation theory, they provided evidence for an S shaped steady state curve bounded by saddle-node bifurcations, with transitions driven by the activity of Cln2 or Cdc20. Indeed, we found that Chen’s model displays such bifurcations when [Cln2] and [Cdc20] are considered as bifurcation parameters.

In accord with the experimental protocol of Cross et al. [10], we consider [Cln2] and [Cdc20] as parameters, and therefore discard Eqn. (A1) and Eqn. (A4-A5) from Chen’s model. We performed bifurcation analysis for the remaining six ODE’s. In Figure 3, we show a combination of two bifurcation diagrams. In the left bifurcation diagram we set [Cln2]=0 and vary [Cdc20], whereas in the right bifurcation diagram we set [Cdc20]=0 and vary [Cln2]. As mass is the same in both cases ($M = 1$), the two stable steady states in Figure 3 represent G1 phase (low Clb2-dependent kinase activity) and S/G2/M phase (high Clb2-dependent kinase activity). Figure 3 shows that increasing [Cln2] drives the transition from G1 into S/G2/M, while activation of [Cdc20] drives the transition from S/G2/M back to G1.

Using AUTO’s facility for computing two-parameter bifurcation diagrams, we extended the saddle-node bifurcations in Figure 3 into the parameter planes spanned by $([Cln2], M)$, $([Cdc20], M)$, and $([Cln2], [Cdc20])$. In Figure 4a, there are multiple steady states inside the cusp-shaped region bounded by the dashed lines, as expected [12]. In Figure 4b, there are two different bistable domains, bounded by dashed and dotted lines, respectively. Where the domains overlap, we found that the control system has five steady states. We found that two different modules independently lead to the bistable domains in Figure 4b. The dashed line curve is due to the Hct1 module of the wiring diagram in Figure 1, whereas the dotted line curve is due to the Sic1 module. Finally, Figure 4c shows the bistable region on the $([Cln2], [Cdc20])$ plane.

V. EFFECTS OF TRANSCRIPTIONAL FACTORS MCM1 AND SBF IN A REDUCED MODEL WITH FOUR ODE'S

Because Eqn. (A1-A20) take into account many known details of cell cycle control, the model is very complex. It is difficult to understand from Eqn. (A1-A20) what are the nonlinearities leading to specific features of the bifurcation diagram shown in Figure 2. To overcome this difficulty, we simplify Chen's model, by defining a core module that retains the main dynamical features of the full set of equations. The reduced model can be useful in understanding the roles of nonlinear feedbacks in the control system.

In Figure 5 we propose a simplified wiring diagram for the budding yeast cell cycle. We discarded from the original wiring diagram the Sic1 and the Clb5 modules and Cdc20's activation, retaining only four ODE's.

$$\frac{d}{dt}[\text{Cln2}] = M(k'_{s,n2} + k''_{s,n2}[\text{SBF}]) - k_{d,n2}[\text{Cln2}], \quad (1)$$

$$\frac{d}{dt}[\text{Clb2}] = M(k'_{s,b2} + k''_{s,b2}[\text{Mcm1}]) - (k'_{d,b2} + (k''_{d,b2} - k'_{d,b2})[\text{Hct1}] + k'''_{d,b2}[\text{Cdc20}])[\text{Clb2}], \quad (2)$$

$$\frac{d}{dt}[\text{Hct1}] = \frac{(k'_{a,t1} + k''_{a,t1}[\text{Cdc20}]) (1 - [\text{Hct1}])}{J_{a,t1} + 1 - [\text{Hct1}]} - \frac{V_{i,t1}[\text{Hct1}]}{J_{i,t1} + [\text{Hct1}]}, \quad (3)$$

$$\frac{d}{dt}[\text{Cdc20}] = (k'_{s,20} + k''_{s,20}[\text{Clb2}]) - k'_{d,20}[\text{Cdc20}], \quad (4)$$

where $[\text{SBF}]$ is given by Eqn. (A13-A14) with $[\text{Clb5}]=0$. $[\text{Mcm1}]$ is given by Eqn. (A11), and $V_{i,t1}$ is given by Eqn. (A15). With the elimination of the dynamics for Cdc20 activation, we define a new parameter in Eqn. (4) $k'_{d,20} = \frac{k_{d,20}k_{a,20}}{k_{a,20} + V_{i,20} + k_{d,20}}$. We note that the results in this section do not change if $k'_{d,20} = k_{d,20}$.

Although Figure 5 is much simpler than Figure 1, Eqn. (1-4) are still quite complex. The most uncertainties arise from the two transcription factors (SBF and Mcm1), which are described by nonlinear Goldbeter-Koshland functions [18, 19]. Their role is to switch solutions from one branch to another. As the effects of the transcription factors can be studied experimentally, we explore their roles via two-parameter bifurcation diagrams. First, by using mass as the primary bifurcation parameter, we computed a one-parameter bifurcation diagram similar to Figure 2. Then, we continued the codimension-one bifurcations into two

parameter domains, using the intensity coefficients of the transcription factors, $k''_{s,n2}$ and $k''_{s,b2}$, as the secondary bifurcation parameters.

Figure 6a shows a two-parameter bifurcation diagram of Eqn. (1-4) on the $(M, k''_{s,n2})$ plane. Saddle-node bifurcations appear in Figure 6a only if $k''_{s,n2} > 0$. A bistable domain is inside the dashed lines. It widens at smaller mass and larger $k''_{s,n2}$. The dot-dashed line showing Hopf bifurcation points continues inside the bistable domain. At $M > 0.8$, the Hopf bifurcation line is accompanied by a cyclic fold curve. These curves eventually coalesce at a larger mass value. Inside the bistable domain the cyclic fold coalesces with a locus of saddle loops (solid line in Figure 6a).

Figure 6b shows a two-parameter bifurcation diagram on the $(M, k_{s,b2})$ plane. A crucial difference between Figure 6a and Figure 6b is the existence of a bistable domain at $k''_{s,b2} = 0$. If $k''_{s,b2} < 0.04$, the effect of [Mcm1] regulation is negligible. But if $k''_{s,b2} > 0.5$, [Mcm1] can destroy bistability. In Figure 6b a Hopf bifurcation line originates from a Bogdanov-Takens bifurcation. This line is accompanied by a line of saddle loops. The saddle loops change stability where the line of cyclic folds coalesces with the line of saddle loops.

We found that Eqn. (A1-A20) display two-parameter bifurcation diagrams similar to Figure 6a-b. Notice from Figure 6b that the domain of bistability is quite independent of the activity of Mcm1, but the existence of the primary Hopf bifurcation in the model is sensitively dependent on the activity of Mcm1.

VI. A SNIC BIFURCATION IN A REDUCED MODEL WITH THREE ODE'S

The eukaryotic cell cycle engine is a highly conserved molecular machine. It is expected that mathematical models of cell cycle controls in different organisms exhibit qualitatively similar dynamics as revealed by similar bifurcation diagrams. But there can be also peculiarities in these models, subject to particular parameter selections. As we mentioned in Section 3, the bifurcation diagram in Figure 2 does not involve a SNIC bifurcation, as seen in bifurcation diagrams of mathematical models for frog eggs and fission yeast [15, 16, 23]. Although this difference is rather subtle and does not contradict any features of cell cycle physiology, we point out that Chen's model Eqn. (A1-A20) can display a SNIC bifurcation for appropriate choice of parameter values (not shown). In this section, we examine SNIC bifurcation in a three variable model.

To further simplify the model, we neglect Cln2 from the wiring diagram in Figure 5. As a result, we have a model with three time-dependent variables,

$$\frac{d}{dt}[\text{Clb2}] = M(k'_{s,b2} + k''_{s,b2}[\text{Mcm1}]) - (k'_{d,b2} + (k''_{d,b2} - k'_{d,b2})[\text{Hct1}] + k'''_{d,b2}[\text{Cdc20}])[\text{Clb2}], \quad (5)$$

$$\frac{d}{dt}[\text{Hct1}] = \frac{(k'_{a,t1} + k''_{a,t1}[\text{Cdc20}])(1 - [\text{Hct1}])}{J_{a,t1} + 1 - [\text{Hct1}]} - \frac{V_{i,t1}[\text{Hct1}]}{J_{i,t1} + [\text{Hct1}]}, \quad (6)$$

$$\frac{d}{dt}[\text{Cdc20}] = (k'_{s,20} + k''_{s,20}[\text{Clb2}]) - k_{d,20}[\text{Cdc20}]. \quad (7)$$

In Eqn.(5-7), [Mcm1] is given by Eqn. (A11), and $V_{i,t1}$ is given by Eqn. (A15). We assume [Cln2] = 0 and $[\text{Clb5}] = \frac{k'_{s,b5}M}{k'_{d,b5}}$ in Eqn. (A15). We also changed the values of some parameters in Table I, as $k'_{s,b5} = 0.06$, $k''_{a,t1} = 1.5$, $k''_{s,20} = 0.07$, $J_{a,mcm} = J_{i,mcm} = 0.01$.

Let $[\text{Clb2}]^0$, $[\text{Hct1}]^0$ and $[\text{Cdc20}]^0$ denote a steady state solution of Eqn. (5-7). Clearly, $[\text{Cdc20}]^0 = \frac{(k'_{s,20} + k''_{s,20}[\text{Clb2}]^0)}{k_{d,20}}$. Substituting this functional relation between [Cdc20] and [Clb2] into Eqn. (5-6), we can think of $([\text{Clb2}], [\text{Hct1}])$ as a two-variable system, susceptible to phase plane analysis. The nullclines of the two variable systems are plotted in Figure 7. From the intersections of these nullclines, we find steady state solutions, $[\text{Clb2}]^0$ and $[\text{Hct1}]^0$, and consequently $[\text{Cdc20}]^0$. Depending on M , the number of intersections varies, but the maximum number of steady states is three.

We study stability of the steady states numerically. In Figure 8 we plot a bifurcation diagram of Eqn. (5-7), with M as the principal bifurcation parameter. At a given M , there can be one stable steady state and two unstable steady states, or a single steady state which can be either stable or unstable. The stable steady states in Figure 8 can coexist with stable limit cycle oscillations. There are two interesting features in this bifurcation diagram: (i) a SNIC bifurcation which arises at $M \approx 2.9$ where a saddle-node coalescence is replaced by limit cycle oscillations, and (ii) byrhythmicity, i.e., coexistence of two stable limit cycle oscillations, for $2.8 < M < 4.9$.

Figure 9 shows a two-parameter bifurcation diagram for Eqn. (5-7). Despite the reduction to just three ODE's, this diagram is quite complex. Multiple steady states are found inside the solid lines. There are three Bogdanov-Takens bifurcations in Figure 9, from which originate three independent loci of Hopf bifurcations, shown by lines in violet. Two cyclic folds associated with the Hopf bifurcations are shown by lines in cyan. Two saddle loops,

shown by green lines, originate at BT_1 and BT_2 , cross the region of bistability, and attach to the right saddle node line at two saddle-node-loop bifurcation points. Between these two saddle-node-loops, we find a SNIC bifurcation, red line in Figure 9.

VII. BIRHYTHMICITY AND EFFECTS OF EXTRINSIC FLUCTUATIONS

Figure 9 shows that the distance between the inner cyclic fold(CF_2) and the SNIC bifurcation varies as $k''_{s,b2}$ changes. In other words, depending on $k''_{s,b2}$, birhythmicity may occur either close to, or far from the START transition (when the stable G1 state gives way to large amplitude stable oscillations). If it happens far away from START, it will not interfere with cell cycle progression. However, if it occurs close to START, as in Figure 8, an interesting question arises. To which stable oscillation (the large amplitude or the small amplitude limit cycles) will the trajectory of motion (see red lines in Figure 2) connect? We found that the trajectory of motion always follows the large amplitude slow oscillations in the three-variable model. We have shown (in a separate publication) that switching between small and large amplitude oscillations is possible when the model takes into account diffusion terms [24]. Here, we demonstrate the effects of noise on the trajectory of motion.

A complex process, such as cell cycle control, is naturally subject to fluctuations from different sources. For instance, stochastic effects due to size and nuclear volume differences at cell division have been studied for fission yeast [25]. Since we know very little about the origin of fluctuations in the cell cycle engine, the simplest way to incorporate random processes into Eqn. (5-7) is to assume that certain extrinsic fluctuations randomly perturb the cell cycle engine. Mathematically, we replace Eqn. (5-7) by Langevin-type equations with multiplicative noise [26, 27],

$$\frac{d}{dt}[\text{Clb2}] = F_{[\text{Clb2}]} + \sqrt{2D_1[\text{Clb2}]} \xi(t), \quad (8)$$

$$\frac{d}{dt}[\text{Hct1}] = F_{[\text{Hct1}]} + \sqrt{2D_2[\text{Hct1}]} \xi(t), \quad (9)$$

$$\frac{d}{dt}[\text{Cdc20}] = F_{[\text{Cdc20}]} + \sqrt{2D_3[\text{Cdc20}]} \xi(t), \quad (10)$$

$$\frac{dM}{dt} = \mu M. \quad (11)$$

where, $F_{[\text{Clb2}]}$, $F_{[\text{Hct1}]}$, $F_{[\text{Cdc20}]}$ are the right hand sides of Eqn. (5-7) and $\xi(t)$ is Gaussian white noise with zero mean and unit variance,

$$\langle \xi(t) \rangle = 0, \quad \langle \xi(t)\xi(t') \rangle = \delta(t - t'). \quad (12)$$

We assume that mass increase is not affected by random fluctuations [27].

We simulated Eqn. (8-11) using standard numerical techniques for stochastic differential equations [28, 29]. In Figure 10 we overplot two different simulations. The dashed lines show time evolutions of M , $[\text{Clb2}]$, $[\text{Cdc20}]$ and $[\text{Hct1}]$ when birhythmicity occurs far from START. In this case, noise does not interfere with mitosis, and cell mass divides each time $[\text{Clb2}]$ drops below 0.1. The solid lines show the case when birhythmicity occurs close to START, as in Figure 8. In this case noise can switch the control system from slow, large amplitude oscillations to fast, small amplitude oscillations. As a result, $[\text{Clb2}]$ does not go below 0.1 and the cell cannot divide. Consequently, mass M grows and the system goes to the stable steady state (see filled diamonds at $M > 3.9$ in Figure 8). Therefore, in the presence of noise, birhythmicity may lead to mitotic arrest.

VIII. DISCUSSION

In this work, we carried out bifurcation analysis of a model of the budding yeast cell cycle, based on earlier work by Chen et al. [9] which successfully accounts for many observed features of proliferating yeast cells. Our results show that, despite a peculiarity in topology of the bifurcation diagram, the budding yeast cell cycle model displays the same basic features previously associated with frog egg and fission yeast models; namely, saddle-node bifurcations associated with stable and unstable oscillations.

We explored bistability and hysteresis in this model by numerical bifurcation analysis. Some of our bifurcation diagrams can be useful for designing new experiments. For instance, our two parameter bifurcation analysis (Figure 4b) suggests that the $[\text{Hct1}]$ and $[\text{Sic1}]$ modules may lead independently to bistable states, and there can be regions in parameter space with three stable steady states, when these two modules operate cooperatively.

We found that a reduced model with four time-dependent variables retains the main characteristics of the bifurcation diagram of Chen's model. This reduction allows us to explore the dominant roles of SBF and Mcm1 transcription factors in budding yeast checkpoint

controls. Our two-parameter bifurcation diagrams (Figure 6) also can be useful in designing experiments for cell cycle controls by transcription factors.

The budding yeast cell cycle model of Chen et al. is parameter rich. Although the parameter set presented in Table I leads to a satisfactory fit of the model to many experimental observations, the choice of parameter values should be further constrained by new biochemical data about the protein-protein interactions and further improved by automatic parameter estimation techniques [30, 31]. On the other hand, different sets of parameters, leading to different bifurcation scenarios, are interesting from a theoretical standpoint. We have proposed a set of parameters for a reduced, three-variable model leading to a SNIC bifurcation.

An interesting feature accompanying the appearance of a SNIC bifurcation in the reduced model is birhythmicity. Birhythmicity has been found in a chemical system [32], but for biological systems, it is known theoretically only [15, 18, 33]. We have shown that in the presence of extrinsic fluctuations, birhythmicity can lead to mitotic arrest. The fact that noise can switch a biochemical system from one stable solution to another is well known (e.g. Ref. [34, 35]), but switching from one stable oscillations to another is a less studied research area. A more systematic study of switching between stable limit cycles is a problem for the future.

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Figure Captions

Fig. 1. Wiring diagram of a budding yeast cell cycle model [9].

Fig. 2. A one-parameter bifurcation diagram of Eqn. (A1-A20) for parameter values in Table I. Solid lines indicate stable steady states. Dashed lines indicate unstable steady states. Solid circles denote the maximum and minimum values of $[\text{Clb2}]_T$ on stable limit cycle oscillations, open circles denote the same for unstable oscillations. The red line shows the trajectory of motion when Eqn. (A1-A20) are supplemented by the mass growth equation $\dot{M} = \mu M$. The cell divides ($M = f \cdot M$) when $[\text{Clb2}]_T$ drops below 0.005.

Fig. 3. Bistability and hysteresis driven by $[\text{Cln2}]$ and $[\text{Cdc20}]$. On the left plane $[\text{Cln2}] \equiv 0$, on the right plane $[\text{Cdc20}] \equiv 0$. Mass is fixed at 1. Filled diamonds show stable steady states, dashed lines show unstable steady states. Dotted lines and arrows indicate the START and FINISH transitions of the hysteresis loop. (START refers to the $\text{G1} \rightarrow \text{S}$ transition, FINISH refers to the $\text{M} \rightarrow \text{G1}$ transition.)

Fig. 4a. Two-parameter bifurcation diagram on the $([\text{Cln2}], M)$ plane. Multiple steady states are found inside the cusp-shaped curve.

Fig. 4b. Two-parameter bifurcation diagram on the $([\text{Cdc20}], M)$ plane. There are two independent pairs of saddle-node bifurcation curves in this figure (dashed curves and dotted curves). Depending on the overlaps of the regions bounded by these curves, the number of steady states varies from I to V.

Fig. 4c. Two parameter bifurcation diagram on the $([\text{Cln2}], [\text{Cdc20}])$ plane. Bistable steady states are found in between the dashed curves.

Fig. 5. Wiring diagram of a reduced model with four-variables.

Fig. 6a. Two-parameter bifurcation diagram of Eqn. (1-4) on the $(k''_{s,n2}, M)$ plane. Loci of saddle node bifurcations (SN) are shown by dashed lines. Other lines trace saddle loop (solid), cyclic fold (dotted), and Hopf (dot-dash) bifurcation points.

Fig. 6b. Two-parameter bifurcation diagram of Eqn. (1-4) on the $(k''_{s,b2}, M)$ plane. Bistable steady states are found inside the SN curve (dashed line). Other lines indicate loci of saddle loops (solid), cyclic folds (dotted), and Hopf bifurcations (dot-dash). The locus of Hopf bifurcations originates from a Bogdanov-Takens point shown by the filled circle.

Fig. 7. Stationary solutions of Eqn. (5-6) can be computed from the intersections of the $[\text{Hct1}]$ nullcline (solid line) and the $[\text{Clb2}]$ nullcline (dashed line). In this plot mass is fixed at $M = 2$. Notice that $[\text{Clb2}] \approx 0.15$ is the region where $[\text{Mcm1}]$ changes abruptly from 0

to 1.

Fig. 8. Bifurcation diagram of Eqn. (5-7). Filled diamonds show stable steady states, dashed lines show unstable steady states. Stable limit cycle oscillations are shown by filled circles, unstable limit cycle oscillations are shown by open circles.

Fig. 9. Two-parameter bifurcation diagram of Eqn. (5-7) on the $(M, k''_{s,b2})$ plane. Bistability is found inside the SN curve (solid line). Three different Hopf bifurcations (violet lines) originate from three Bogdanov-Takens bifurcation points shown by filled circles at BT_1 , BT_2 and BT_3 . Cyclic folds are shown by lines in cyan, saddle loops by lines in green, and the red solid line shows SNIC bifurcations. SL_3 , which runs next to HB_3 , is not shown on the diagram. CF_2 runs from a degenerate Hopf bifurcation on HB_1 to a degenerate Hopf bifurcation on HB_2 . CF_1 runs from a degenerate saddle loops on SL_1 to a degenerate saddle loop on SL_2 (not shown), crossing over HB_1 on the way. Where CF_1 and CF_2 run very close together, only CF_2 is plotted on the figure.

Fig. 10. Stochastic simulations of Eqn. (8-11). Dashed lines show a case when birhythmicity occurs far from the SNIC bifurcation. In this case, noise does not interfere with cell cycle progression. Solid lines show the case when birhythmicity occurs close to the SNIC bifurcation, as in Figure 8. In the presence of noise, the latter case leads eventually to mitotic arrest. Parameters are: $D_1 = D_2 = D_3 = 3.75 \cdot 10^{-5}$. Solid lines for $k''_{s,b2} = 0.05$, dashed lines for $k''_{s,b2} = 0.06$.

APPENDIX A: MODEL FOR BUDDING YEAST CELL CYCLE

$$\frac{d}{dt}[\text{Cln2}] = M(k'_{s,n2} + k''_{s,n2}[\text{SBF}]) - k_{d,n2}[\text{Cln2}], \quad (\text{A1})$$

$$\frac{d}{dt}[\text{Clb2}]_T = M(k'_{s,b2} + k''_{s,b2}[\text{Mcm1}]) - (k'_{d,b2} + (k''_{d,b2} - k'_{d,b2})[\text{Hct1}] + k'''_{d,b2}[\text{Cdc20}])[\text{Clb2}]_T, \quad (\text{A2})$$

$$\frac{d}{dt}[\text{Hct1}] = \frac{(k'_{a,t1} + k''_{a,t1}[\text{Cdc20}])(1 - [\text{Hct1}])}{J_{a,t1} + 1 - [\text{Hct1}]} - \frac{V_{i,t1}[\text{Hct1}]}{J_{i,t1} + [\text{Hct1}]}, \quad (\text{A3})$$

$$\frac{d}{dt}[\text{Cdc20}]_T = (k'_{s,20} + k''_{s,20}[\text{Clb2}]) - k_{d,20}[\text{Cdc20}]_T, \quad (\text{A4})$$

$$\frac{d}{dt}[\text{Cdc20}] = k_{a,20}([\text{Cdc20}]_T - [\text{Cdc20}]) - (V_{i,20} + k_{d,20})[\text{Cdc20}], \quad (\text{A5})$$

$$\frac{d}{dt}[\text{Clb5}]_T = M(k'_{s,b5} + k''_{s,b5}[\text{MBF}]) - (k'_{d,b5} + k''_{d,b5}[\text{Cdc20}])[\text{Clb5}]_T, \quad (\text{A6})$$

$$\frac{d}{dt}[\text{Sic1}]_T = k'_{s,c1} + k''_{s,c1}[\text{Swi5}] - (k_{d1,c1} + \frac{V_{d2c1}}{J_{d2,c1} + [\text{Sic1}]_T})[\text{Sic1}]_T, \quad (\text{A7})$$

$$\begin{aligned} \frac{d}{dt}[\text{Clb5}|\text{Sic1}] &= k_{as,b5}[\text{Clb5}][\text{Sic1}] - \\ &- (k_{di,b5} + k'_{d,b5} + k''_{d,b5}[\text{Cdc20}] + k_{d1,c1} + \frac{V_{d2c1}}{J_{d2,c1} + [\text{Sic1}]_T})[\text{Clb5}|\text{Sic1}], \end{aligned} \quad (\text{A8})$$

$$\begin{aligned} \frac{d}{dt}[\text{Clb2}|\text{Sic1}] &= k_{as,b2}[\text{Clb2}][\text{Sic1}] - \\ &- (k_{di,b2} + (k'_{d,b2} + (k''_{d,b2} - k'_{d,b2})[\text{Hct1}] + k'''_{d,b2}[\text{Cdc20}]) + k_{d1,c1} + \frac{V_{d2c1}}{J_{d2,c1} + [\text{Sic1}]_T})[\text{Clb2}|\text{Sic1}], \end{aligned} \quad (\text{A9})$$

$$V_{d2,c1} = k_{d2,c1}(\epsilon_{c1,n3}[\text{Cln3}]^* + \epsilon_{c1,k2}[\text{Bck2}] + [\text{Cln2}] + \epsilon_{c1,b5}[\text{Clb5}] + \epsilon_{c1,b2}[\text{Clb2}]), \quad (\text{A10})$$

$$[\text{Mcm1}] = G(k_{a,mcm}[\text{Clb2}], k_{i,mcm}, J_{a,mcm}, J_{i,mcm}), \quad (\text{A11})$$

$$[\text{Swi5}] = G(k_{a,swi}[\text{Cdc20}], k'_{i,swi} + k''_{i,swi}[\text{Clb2}], J_{a,swi}, J_{i,swi}), \quad (\text{A12})$$

$$[\text{SBF}] = [\text{MBF}] = G(V_{a,sbf}, k'_{i,sbf} + k''_{i,sbf}[\text{Clb2}], J_{a,sbf}, J_{i,sbf}), \quad (\text{A13})$$

$$V_{a,sbf} = k_{a,sbf}([\text{Cln2}] + \epsilon_{sbf,n3}([\text{Cln3}]^* + [\text{Bck2}]) + \epsilon_{sbf,b5}[\text{Clb5}]), \quad (\text{A14})$$

$$V_{i,t1} = k'_{i,t1} + k''_{i,t1}([\text{Cln3}]^* + \epsilon_{i,t1,n2}[\text{Cln2}] + \epsilon_{i,t1,b5}[\text{Clb5}] + \epsilon_{i,t1,b2}[\text{Clb2}]), \quad (\text{A15})$$

$$[\text{Clb2}]_T = [\text{Clb2}] + [\text{Clb2}|\text{Sic1}], \quad (\text{A16})$$

$$[\text{Clb5}]_T = [\text{Clb5}] + [\text{Clb5}|\text{Sic1}], \quad (\text{A17})$$

$$[\text{Sic1}]_T = [\text{Sic1}] + [\text{Clb2}|\text{Sic1}] + [\text{Clb5}|\text{Sic1}], \quad (\text{A18})$$

$$[\text{Bck2}] = M[\text{Bck2}]^0, \quad (\text{A19})$$

$$[\text{Cln3}]^* = [\text{Cln3}]_{\max} \frac{MD_{n3}}{J_{n3} + MD_{n3}}. \quad (\text{A20})$$

TABLE I: Kinetik constants for the budding yeast model

| Rate constants(min^{-1}) | | | |
|--|------------------------------|----------------------------|------------------------|
| $k'_{s,b5} = 0.006$ | $k''_{s,b5} = 0.02$ | $k'_{d,b5} = 0.1$ | $k''_{d,b5} = 0.25$ |
| $k'_{s,n2} = 0$ | $k''_{s,n2} = 0.05$ | $k_{d,n2} = 0.1$ | |
| $k'_{s,b2} = 0.002$ | $k''_{s,b2} = 0.05$ | | |
| $k'_{d,b2} = 0.010$ | $k''_{d,b2} = 2$ | $k'''_{d,b2} = 0.05$ | |
| $k'_{s,c1} = 0.020$ | $k''_{s,c1} = 0.1$ | $k_{d1,c1} = 0.01$ | $k_{d2,c1} = 0.3$ |
| $k_{as,b2} = 50$ | $k_{as,b5} = 50$ | $k_{di,b2} = 0.05$ | $k_{di,b5} = 0.05$ |
| $k'_{s,20} = 0.005$ | $k''_{s,20} = 0.06$ | $k_{d,20} = 0.08$ | |
| $k_{a,20} = 1$ | $k'_{i,20} = 0.1$ | $k''_{i,20} = 10$ | |
| $k'_{a,t1} = 0.04$ | $k''_{a,t1} = 2$ | $k'_{i,t1} = 0$ | $k''_{i,t1} = 0.64$ |
| $k_{a,sbf} = 1$ | $k_{a,mcm} = 1$ | $k_{a,swi} = 1$ | $k_{i,mcm} = 0.15$ |
| $k'_{i,sbf} = 0.5$ | $k''_{i,sbf} = 6$ | $k'_{i,swi} = 0.3$ | $k''_{i,swi} = 0.2$ |
| Characteristic concentrations(dimensionless) | | | |
| $[\text{Cln3}]_{\text{max}} = 0.02$ | $[\text{Bck2}]^0 = 0.0027$ | $J_{d2,c1} = 0.05$ | |
| $J_{a,sbf} = J_{i,sbf} = 0.01$ | $J_{a,mcm} = J_{i,mcm} = 1$ | | |
| $J_{a,swi} = J_{i,swi} = 0.1$ | $J_{a,t1} = J_{i,t1} = 0.05$ | | |
| Kinase efficiencies(dimensionless) | | | |
| $\epsilon_{c1,n3} = 20$ | $\epsilon_{c1,k2} = 2$ | $\epsilon_{c1,b2} = 0.067$ | $\epsilon_{c1,b5} = 1$ |
| $\epsilon_{i,t1,n2} = 1$ | $\epsilon_{i,t1,b2} = 1$ | $\epsilon_{i,t1,b5} = 0.5$ | |
| $\epsilon_{sbf,n3} = 75$ | $\epsilon_{sbf,b5} = 0.5$ | $V_{i,20} = 0.05$ | |
| Other parameters | | | |
| $f = 0.433$ | $J_{n3} = 6$ | $D_{n3} = 1$ | $\mu = 0.005776$ |

Golldbeter Koshland function:

$$G(a, b, c, d) = \frac{2ad}{b - a + bc + ad + \sqrt{(b - a + bc + ad)^2 - 4ab(b - a)}} \quad (\text{A21})$$

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- [36] we adopted following notations: ABC denotes a gene, ABC implies a protein, and $[ABC]$ denotes a concentration for protein ABC .



